RESEARCH ARTICLE

Synthesis and Preliminary Antimicrobial Activity Evaluation of New Amide Derivatives of 2-aminobenzothiazole

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ABSTRACT

Heterocyclic chemistry takes part a crucial role in drug development. Accordingly, novel heterocyclic compounds of 2-aminobenzthiazole were synthesized and tested against the microbial activity. Carbamoyl chloride of benzothiazole (compound M) was prepared, then coupled with other heterocyclic thiol derivatives through s-alkylation reaction to give compounds (MH1, MH2, MZ1, MZ2). Characterization of these compounds was done using Fourier Transform Infrared Spectroscopy (FTIR), and ^{1}HNMR analyses. The antimicrobial results showed that all compounds at concentration 250 µg/mL, except compound MH1, have moderate activity against "the gram-negative *Escherichia coli*". Furthermore, compounds (MZ1 and MZ2) are moderately active against "the gram-positive *Staphylococcus epidermidis*". Finally, only compound MZ1 have moderate activity against "the gram-positive *Klebsiella pneumonia*".

Keywords: Antimicrobial, Benzothiazoles, Heterocyclic compounds, S-alkylation, *Staphylococcus epidermidis*.

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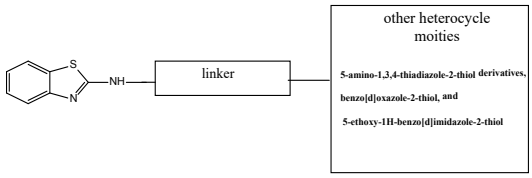
INTRODUCTION

Heterocyclic chemistry plays an essential role in the development of biologically active drugs. Many biological active drugs are made up of hetero-atoms. As a result, most of the pharmaceutical molecules exhibit biological activity due to the heterocyclic fragments.[1] Numerous heterocyclic moieties can be considered as a beneficial structure. Mostly, nitrogen-containing heterocycles or various combinational set of nitrogen atoms, sulphur, and oxygen are in different positions of five- or six-membered rings.[2]

Among these heterocycles, benzothiazole has particular and wide use in experimental drugs. Special focus in synthetic and pharmaceutical chemistry study has been paid to 1,3-benzothiazole derivatives because of their powerful and considerable pharmacological activities.[3] It is known that benzothiazole consists of two fused rings, one six-membered (benzene) fused with one five-membered ring (thiazole). Both rings are responsible for therapeutic activity.[4] It is also known that a small change in the heterocyclic moiety of a drug molecule can result in a large therapeutic change.[5]

Many researchers have synthesized biologically active 2-aminobenzthiazole derivatives. They showed "anti-tubercular,[6] anthelmintic,[7] antibacterial,[8] anticonvulsant,[9] anticancer,[10,11] and anti-inflammatory[12] activity. Bolelli's team created new 2-(4-aminophenyl) benzothiazole derivatives that



were tested against microbial activity. The results showed that the new compounds have a wide range of activity upon the investigated microorganisms.[13] Balram Soni *et al*. synthesized and tested benzthiazole derivatives for antimicrobial activity. They discovered that all of the derivatives exhibited total inhibition at varying minimum inhibitory concentrations.[14]

Nowadays, antimicrobial medications are rapidly become resisted by pathogenic microorganisms. As a result, one of the most significant anti-microbial fields of study has started to emerge: the progress of innovative chemicals to fight resistant microbes.[15,16] Thus, it was aimed to synthesize 2-amino-benzothiazole molecule linked with other well-known biological active molecules using a suitable linker.

EXPERIMENTAL

Chemicals and Instruments

Chemicals

All chemicals were reagent grade and obtained from (Hyperchem), 2-mercapto-benzoxazole (fluka, Germany),

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5-ethoxy-2-mercapto-benzimidazole (Sigma-Aldrich, America). Reaction monitoring was done with thin layer chromatographic (TLC) technique using Silica gel pre-coated plates having F254 indicator. All synthesized derivatives were characterized by spectroscopic analysis (Fourier-transform IR spectra spectrometer, Shimadzu, and (Proton nuclear magnetic resonance (^{1}H-NMR) at 500 MHz using "DMSO-d6".

General Synthetic Procedures

Synthesis of Compound M

Compound M was synthesized with slight modification to reported reference by dissolving (1 gm, 6.65 mmole) of 2-amino benzothiazole in dry chloroform (10 mL) on an ice bath. Then, 1.85 mL of triethylamine was added drop by drop with continuous stirring and simultaneous dropwise addition of 1.1 mL of chloro-acetyl chloride. The stirring was continued overnight, where the solution's color turned from bright yellow to pink to beige suspension. After that, the mixture's volume was reduced, filtered. The precipitated residue was dried after washing with distilled water.[17]

N-(benzo[d]thiazol-2-yl)-2-chloroacetamide: Pink powder, yield 58%; melting point= 136–140ºC; FTIR (cm^{-1}): 3186 (amide N-H, str.), 3059 (aromatic-H, str.), 2924 (CH_2, str.), 1697 (amide carbonyl str.), 1570–1442 (C=C-C aromatic, str.). ^{1}HNMR (DMSO) (δ, ppm): 12.73 (s, 1H, amide NH), 7.31- 8.01 (4H, m, C-H Aromatic proton), 4.48 (s, 2H, CH_2).

Synthesis of Compound H1

Carbon disulfide (1.055 g, 13.88 mmole) was gradually added to thiosemicarbazide suspension (0.44 g, 4.77 mmole) and anhydrous $NaCO_3$ (0.25 gm, 2.33 mmole) in absolute ethanol (10 mL). The resulted mixture was allowed to reflux for 6 hours. After that, the mixture was allowed to cool and filtered. Cold water was added to the evaporated filtrate, followed by acidification with concentrated HCl drop by drop, resulting in a white-yellowish suspension, that is filtered and finally washed with D.W to get the product.[18]

5-Amino-1,3,4-thiadiazole-2-thiol: bright yellow powder, 70% yield, melting point: 231–233°C. FTIR (cm^{-1}): 3325 and 3244 (NH of amine, stretching), 1550 (C=N stretching).

Synthesis of Compound H2

Compound H2 was synthesized with slight modification to reported reference by dissolving (0.1 gm, 0.75 mmole) of compound H in dry CH_2Cl_2 (10 mL). Then, on an ice bath, 0.16 mL (1.125 mmol) triethylamine and 0.1 mL (1.5 mmol) acetyl chloride was added drop by drop with continuous stirring. Overnight, the stirring continued. The resulted suspension was filtered after reducing the solvent volume. Finally, the residue was washed with D.W to obtain the final product.[19]

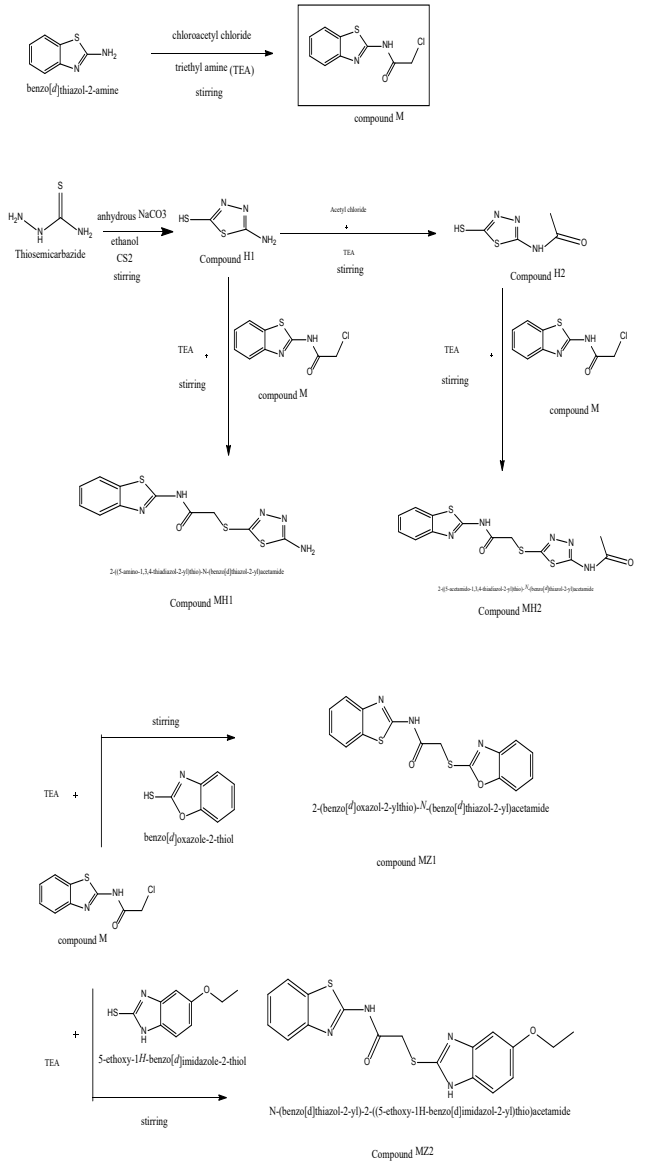
N-(5-mercapto-1,3,4-thiadiazol-2-yl) acetamide: white solid, yield 59%; melting point = 287–290◦C; FTIR (cm^{-1}): 3151 (NH of amide, str.), 3059 (aromatic H, str.), 2970–2877(CH_3 str.), 1651 (C=O amide carbonyl str.), 1573–1442 (C=C aromatic, str.).

Synthesis of compound MH1 and MH2

In dry methanol with a few drops of triethylamine, an equimolar amount of compound M (0.1 g, 0.44 mmol) was added to compound H (0.06g, 0.45 mmol) or compound H2 (0.077 gm, 0.45 mmole) and refluxed for 4 hours. The solvent was removed to its third, filtered, and dried in an oven at 60 C.[20]

2-((5-amino-1,3,4-thiadiazol-2-yl) thio)-N-(benzo[d]thiazol-2-yl) acetamide (MH1): white powder, yield 62%; melting point= 272–275ºC; FTIR (cm^{-1}): 3433 & 3402 (NH_2, str.), 3263 (amide H, str.), (3070 aromatic H, str.), 2947-2931 (CH_2 stre.), 1678 (C=O amide carbonyl str.), 1577–1438 (aromatic C=C-C, str.). ^{1}HNMR (DMSO) (δ, ppm): 12.62 (s, ^{1}H, NH of amide), 7.32 (s, 2H, NH of amine), 7.44-8.01 (4H, m, aromatic-H), 4.18 (s, 2H, CH_2).

2-((5-acetamido-1,3,4-thiadiazol-2-yl)thio)-N-(benzo[d] thiazol-2-yl)acetamide(MH_2): Off-white powder, yield 67%; melting point= 307–309°C; FTIR (cm^{-1}):3170 broad (N-H of NH-C=OCH_3 and NH-C=OCH_2), 3051 (aromatic-H, str.), 2943, 2866 (CH_3 str.), 2904, 2804 (CH_2 str.), 1685 broad (C=O amide



Scheme 1: Synthetic pathway for compounds M, MH1, MH2, MZ1, MZ2

Table 1: *In-vitro* antibacterial activity and anttifungal activity

		S. aureus	*B. epidermidis*	*E. coli*	*K. pneumoniae*	*C. albicans*
Compound Name	*Conc. µg/mL*	*Zone of inhibition(mm)*				
Compound MH1	250	-	-	-	-	-
Compound MH2	250	-	-	11	-	-
Compound MZ1	250	-	12	12	12	-
Compound MZ2	250	-	13	11	-	-
Amoxicillin	10	39	17	37	-	33
Streptomycin	300	19	24	20	15	31
Fluconazole	25	18	18	14	10	14
DMSO	-	-	-	-	-	-

(-) = No activity- slightly active (zone of inhibition between moderately active (zone of inhibition between 10-20 nun), highly active (zone of inhibition more Than 20 mm)

of NH-C=OCH_3 and NH-C=OCH_2), 1600–1423 (aromatic C=C-C, str.).

^{1}HNMR (DMSO) (δ, ppm): 12.69 (s, ^{1}H, NH-C=OCH_2), 12.61 (s, ^{1}H, NH-C=OCH_3), 7.31-8.00 (4H, m, Ar-H), 4.37 (s, 2H, CH2), 2.18 (s, C-H, CH3).

Synthesis of Compound MZ1

Benzo[d]oxazole-2-thiol (0.0668 gm, 0.442 mmol) in water stirred for a while with triethyl amine (0.062 mL, 0.442 mmol), then (0.1 gm, 0.442 mmol) of compound M in (3 mL) of dimethylformamide was gradually added. The resulted mixture was allowed to be stirred overnight. Then filtered, and the product on filter paper was washed with D.W and recrystallized from ethanol.[21]

2-(benzo[d]oxazol-2-ylthio)-N-(benzo[d]thiazol-2-yl) acetamide (MZ1)

Pale yellow powder, yield 50%, melting point 171°C, FTIR (υ= cm^{-1}): 3363 (NH) of 2° amide; 3132 (C-H aromatic, stretching); 2866 (CH) stretch of CH_2; 1689 (C=O) amide; 1643 (C=N); 1597, 1562, 1477 (C=C) aromatic. ^{1}HNMR (δ, ppm): 12.77 (s, 1H, NH of amide), 7.32 (s, 2H, NH of amine), 7.34-8.02 (8H, m, Ar-H), 4.52 (s, 2H, CH2).

Synthesis of Compound MZ2

To a mixture of 5-ethoxy-1H-benzo[d]imidazole-2-thiol (0.085 gm, 0.442 mmol) in 15 mL of water and triethyl amine (0.062 mL, 0.442 mmol), compound M (0.1 gm, 0.442 mmol) in (3 mL) of dimethylformamide was gradually added. The mixture was stirred overnight, filtered, and the product on filter paper was washed with D.W and recrystallized from ethanol.[21]

N-(benzo[d]thiazol-2-yl)-2-((5-ethoxy-1H-benzo[d]imidazol-2-yl)thio)acetamide (MZ2): Brown powder, yield 56%, melting point 125°C; IR (cm^{-1})**:** 3321 (NH) of 2° amide and imidazole; 3082 (C-H aromatic, stretching); 2978, and 2873 (CH) asymmetric and symmetric stretch of CH_3 and CH_2; 1670 (C=O) amide; 1630 (C=N); 1597, 1562, 1500 (C=C) aromatic.

^{1}HNMR (δ, ppm): 12.36 (s, 1H, amide N<u>H</u>), 12.76(s, 1H, of imidazole N<u>H</u>). 6.72-8.02 (7H, m, Aromatic-H), 3.99(s, 2H, CO-CH_2-S), 4.4(q, 2H, O-CH_2-methyl), 1.33 (t, 3H, CH_2-C<u>H</u>$_3$).

RESULTS AND DISCUSSION

Chemistry

Compounds (M, H1, H2, MH1, MH2, MZ1, MZ2) were prepared according to the scheme 1. The path of synthesis begins with creating the core molecule (compound M) by reacting chloroacetyl chloride with 2-aminobenzthiazole. On the one hand, compound M was s-alkylated with (compound H1) and (compound H2) to give (compound MH1), and (compound MH2), respectively. Conversely, compound M was coupled via s-alkylation with benzo-fused unsaturated nitrogen heterocycles derivatives giving (compound MZ1), and (compound MZ2).

Antimicrobial Study

The Antimicrobial activity was done using a well diffusion method. Two types of "Gram-negative bacteria *K. pneumonia* and *E. Coli* and two types of gram-positive bacteria *S. aureus* and *S. epidermidis* were used for testing the *in-vitro* antibacterial activity, and the fungi species *C. albicans*" for testing the *in-vitro* antifungal activity, using amoxicillin and streptomycin as and fluconazole as standards while DMSO as a solvent. The results depicted in Table 1 refer that all compounds at concentration 250 µg/mL, except compound MH1, have moderate activity against "the gram-negative *E. coli*". Furthermore, compounds (MZ1 and MZ2) are moderately active against "the gram-positive *S. epidermidis*". Finally, only compound MZ1 has moderate activity against "the gram positive *K. pneumonia*".

CONCLUSIONS

To sum up, 2-aminobenzthiazole was linked with biological important compounds (5-amino-1,3,4-thiadiazole-2-thiol derivatives, benzo[d]oxazole-2-thiol, and 5-ethoxy-1H-benzo[d] imidazole-2-thiol) to give new heterocyclic compounds. It was found that all compounds, except compound MH1, were found to have moderate activity on *E. coli* bacterium at concentration of 250 µg/mL. And only compound MZ1 is moderately active against *K. pneumonia*. Finally, compounds (MZ1 and MZ2) are moderately active against *S. epidermidis*.

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